

# ON THE SO-CALLED INTESTINAL GLANDS IN NECTURUS MACULATUS

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## *Introduction.*

Imbedded in the submucosa and connected to the mucosa in the intestines of *Necturus* are groups of cells whose function has not been determined. The finer structure and function of these groups of cells constitute the object of the present work.

Groups of cells of similar character have been described in *Proteus*, the European Salamander, Newt, Triton and *Amblystoma*, but have never been mentioned in connection with other animals. It is of interest to note that forms as closely allied as *Rana* do not possess these groups of cells, nor, according to a recent paper by A. M. Reese, does the Alligator. Since the forms mentioned above represent two of the three suborders of *Urodeles* it is probable that they are characteristic of *Urodeles* only.

The function of these groups of cells may be similar in the different animals which possess them, but two views have been held as to their function. One view is that they are glands discharging into the intestine. This other view is that they serve as proliferation centers where the epithelial cells which constitute the musoca are produced.

Various names, more or less suggestive of function, have been applied to these structures, such for example as "gland," "Sprossen" (sprout), "Bud," "Zapfen" (spigot) and "Ersatzzellen" (compensation cells). It is clear that it is not advisable to use for these structures, a name which suggests a function until their function has been ascertained. So, for the sake of convenience, I shall use the term "protuberance" inasmuch as it does not suggest a function.

In the present work I shall confine myself to these protuberances in *Necturus*.

## *Review of Literature.*

Hoffman (1878) was probably the first writer to mention these protuberances in *Necturus*. I have not been able to see Hoffman's work but according to B. F. Kingsbury, Hoffman described these

protuberances in *Necturus* as glands and "speaks of their almost circular opening upon the surface epithelium of the intestine."

Oppel in 1889 saw protuberances in the intestinal submucosa in *Proteus anguineus* and pronounced them glands (Drüsen). He noted that mitotic cells were abundant in the structures and in the intestinal mucosa in the immediate vicinity of the structures.

Protuberances in the intestinal mucosa of *Triton* were described in 1892 by Bizzozero. He referred to them as groups of compensation cells (Ersatzzellen) and sprouts (Sprossen). He said that cells proliferate not only at the base of the mucosa but press the wall of the mucosa out in places to form sprouts. For saying that these sprouts are epithelial, he gave the following reasons, which are summarized from the translation; (1) their general character and constitution are those of epithelial tissue, (2) cells can be seen in all stages of transformation from the cubical cells of the sprout to the columnar cells of the mucosa. He remarked that mucous granules can be seen between the epithelial elements and that a great many mitoses are present in the sprouts.

In Salamander protuberances have been described by Nicholas (1894) who called them proliferation buds (bourgeons germinatifs). Nicholas said that they represent cell proliferation centers, and that they could not be glands inasmuch as they possess no lumen. He said that dividing cells were to be seen only in the buds (bourgeons) or in the surface epithelium near the neck of a bud.

Kingsbury in 1894 working on the enteron of *Necturus* regarded these protuberances as glands. He wrote "my study of them in *Necturus* would, however, lead me to regard them as glands. The arrangement of the cells as if surrounding a lumen, and indeed a lumen itself, which Nicholas declared did not exist in these structures, could be seen upon almost all of my sections in some of the glands. I was unable, however, quite satisfactorily to demonstrate the existence of a neck, although it seemed in numerous glands to be quite well indicated."

The work of Bizzozero was resumed and confirmed by Sacerdotti in 1896. The latter also working on *Triton* sought to ascertain the relation between the period of cell division and the period of mucous secreting activity. He determined that karyo-

kinetic forms were assumed by cells which had already begun to secrete mucous, that these karyokinetic cells are found in the deeper parts of the epithelial layer but more especially in the epithelial spigots (Zapfen) pressing into the connective tissue. He added that occasionally and exceptionally karyokinetic cells were seen near the surface of the epithelium. Sacerdotti explained their presence in that region by saying that the cells in the animal concerned were possessed of very great growth capacity and thus certain mitotic cells had been forced out of the epithelial spigots and had reached the surface while still in mitosis.

Bates in 1904 described protuberances in the American *Amblystoma* and remarked that their structure would suggest glandular activity yet said he was not able to demonstrate the existence of a lumen.

#### *Technique.*

Inasmuch as the main object of my work was to determine the finer structure of these protuberances and with a view to distinguish whether they were glands or not, I endeavored to obtain a technique which would give to glandular secretions a differential coloration. For this purpose some of Dr. R. R. Bensley's special methods for glands were used.

Mallory's triple stain (Guyer, *Animal Microcology*, p. 172) was used extensively. It proved to be especially rapid and practical for general work. It gave a conspicuous differential coloration to connective tissue and epithelial tissue.

Weigert's Hematoxylin (Guyer, p. 178) proved to be very effective for preparing slides for the purpose of studying mitosis and details of nuclear structure.

Some of the animals were killed very soon after being brought into the laboratory, others were kept in an aquarium for a few months. Most of the fixing was done in Gilson's fluid but some fixing was done in Bensley's Acetic Osmic Bichromate fluid.

#### *Description.*

These protuberances in *Necturus* are located for the most part at the peripheral or outer parts of the folds. Occasionally one is found on the side of a fold a short distance from the peripheral bend. In one animal, however, out of over twelve which were

studied, in which the intestinal mucosa was more abundant and more folded, I saw a few of these protuberances occupying positions at the very central part of the sections at the central apices of some of the folds.

As for the distribution of these protuberances throughout the length of the intestine, I found that they are more abundant anteriorly than they are posteriorly. To ascertain this, I made a count of the number of protuberances that appeared in cross sections in the respective regions. The count involved twenty slides from each region from about five different animals. The results of the count are here summarized.

Region	Maximum No.	Minimum No.	Average No.
Duodenal region	109	43	70.3
Middle intestine	104	45	66.6
Rectum	75	37	52.6

Thus it is seen that the number of protuberances decreases with distance from the pylorus.

The size of these protuberances varies in diameter from about 50 micra to about 127 micra. In length they vary from about 127 micra to about 293 micra. These measurements made with a stage micrometer and an ocular micrometer, are the extreme measurements of a great many taken.

The shape of these protuberances is naturally variable but it is usually possible to distinguish three parts. (1) the body, that part of the structure which lies wholly outside the mucosa, (2) the mucosal portion, or the portion that lies wholly within the mucosa and (3) the neck which connects the body and the mucosal portion. I shall describe each part separately and in detail.

The body is commonly roughly spherical in shape but is also frequently columnar or conical with the point more or less blunt. The body is also frequently branched. These structures are invariably connected to the mucosa. In mounted sections one sees many bodies apparently not connected to the mucosa but by tracing such bodies through serial sections a connection with the mucosa could always be demonstrated.

Those protuberances that are situated quite at the peripheral ends of the folds almost always extend into the submucosa in a direction perpendicular to the mucosa. If however the protuberances are situated on the sides of the folds, as related above, the body part bends over so as to lie close to the mucosa. In other words, the body parts of the protuberances always extend in a direction radiating from the center of the intestine.

The cells in the body portions are polygonal in shape. The nuclei are more or less spherical and occupy nearly all of the cell. I studied carefully the narrow zone of protoplasm which surrounds the nucleus in cross sections to see if I could discover any granules the presence of which would suggest a secretory activity, but none of my preparations showed any.

I paid particular attention to the arrangement of cells within the body, and was unable to demonstrate a universal definite order of arrangement. This is contrary to the findings of Kingsbury, who stated that the cells were arranged as if surrounding a lumen, and added that a lumen itself could be seen in some of his sections. I was unable to discover a lumen nor did I find an arrangement of cells that would suggest the presence of a lumen.

Therefore, I have concluded that these protuberances cannot be glands. I consider the presence or absence of a lumen a sufficient criterion on which to base such a conclusion, for if these structures were glands the existence of a duct through which the secretions might pass and a corresponding arrangement of cells would be necessary.

The mucosal portion is that portion which extends into the epithelial mucosa. It commonly spreads out within the mucosa so that cross sections appear fan shaped. There is a gradual transition in the shape of the cells of the body of the protuberance and the columnar cells of the mucosa. The transition takes place in the mucosal portion of the protuberance. The cells in the mucosal portion of the protuberance become more and more elongate and are arranged in crescentic layers in optical section, as if suspended hammock-like from the outer boundary of the mucosa. The farther the cells are from the center of the protuberance, the more columnar they are. The nuclei become more and more oval.



The neck of the protuberance is the portion at the peripheral boundary of the mucosa, which connects the body of the protuberance to the mucosal portion. It is almost always somewhat constricted.

Cells in various stages of mitosis are frequent in all parts of the protuberance. To determine the ratio of mitoses present to the total number of cells in the protuberances, I made a count involving several thousands of cells and calculated that the ratio of mitotic cells to the total number of cells is one to forty-one. There are an average number of 39 cells in a section of a protuberance.

Thus there is an average of about one mitotic cell in a protuberance. In some protuberances I saw three cells in mitosis while, on the other hand, there were many sections of protuberances that did not show a single mitosis.

It is significant that not one case of mitosis was seen in the mucosa at any considerable distance from a protuberance.

Upon these facts I have concluded that cells which are to compose the intestinal mucosa in *Necturus*, are formed in these protuberances. It would seem that the cells are forced through the neck of the protuberance, while perhaps sometimes still in mitotic condition.

In the mucosal portion of the protuberance, the cells appear to be forced in a lateral direction along the mucosa. Since mitotic cells are not found at points along the mucosa excepting near the protuberances, it may be concluded that the intestinal mucosal cells do not divide after they have become functional cells.

According to this view, these protuberances in *Necturus* are cell proliferation centers for the mucosa as was concluded for some other Urodeles by Bizzozero and Nicholas.

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Camera lucida drawing of a portion of a cross section of the mucosa of the mid intestine of *Necturus maculatus* showing protuberance P, lumen L, neck N, mitotic cells M. The cell walls were not drawn by means of a camera lucida. Spencer Ob. 10X, Oc. 4.